SUGGESTED MODIFICATIONS OF THE SALISBURY STOMATA INDEX DEVISED FROM A STUDY OF *STANHOPEA* (ORCHIDACEAE)

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ABSTRACT

Calculations of the stomata index have resulted in wide variances in indices both single and between plants. Modification of the formula is suggested, including a metl of more accurately estimating stomata and subsidiary cells, with accuracy capable of be checked by the use of an identity involving adaxial and abaxial leaf surfaces.

RESUMEN

Los cálculos del índice estomático dieron variaciones amplias, tanto en un indivic como entre diferentes plantas. Se sugiere la modificación de la fórmula, para incluir método de cálculo más seguro de estimación de estomas y células subsidiarias, con u precisión capaz de ser comprobada por el uso de una identidad que abarque las superfic del haz y el envés de las hojas.

INTRODUCTION

When initiating our investigation of the role of stomata in water loss leaves of members of the genus *Stanhopea* (Orchidaceae) we approached t problem by initially attempting to determine the stomatal density i given area of leaf surface. However, results were inconclusive despite tempts to ameliorate variations by recording stomatal frequency in ter of the stomata to the proportion of epidermal cells as suggested by St (1965). Sinclair (1990) expressed preference for the use of the stomata dex (SI) of Salisbury (1928) over the mere determination of stomata d sity. This index is arrived at by adding the number of epidermal cells to number of stomata, dividing this into the number of stomata, and mu plying the result by 100.

Stace (1965) as well as Metcalf and Chalk (1979), working with dico ledonous material, found this index to be quite variable both in sin plants and between plants. The rationale given for this variability was t although a leaf developing under water stress retains the total numbe epidermal cells, the cells are smaller, with the result that stomata densit ncreased in the stressed leaf. Conversely, Rowson (1943 a, b, 1946), ci

in Metcalf and Chalk (1979), working with the genus *Cassia* (Fabaceae), concluded that species of the same genus may be differentiated by means of the stomatal index. Rowson also found that the stomatal index: (a) did not vary significantly at different positions upon the leaf surface; (b) was independent of leaf size and habitat; and (c) was the same for different varieties within a species. Working with monocotyledons, Singh and Singh (1974) report an SI range of 2.0–17.0 for seven species of epiphytic orchids, and Boros (1980) reports 2.5–30.3 for 11 species of terrestrial orchids.

Rasmussen (1987) mentions *Stanhopea* only casually, stating that a species (*S. tigrina*) was included in a summary by Strasburger (1866–67). Similarly, reviewing water relations in orchids, Sinclair notes the genus once as a reference by Link (1849) who saw spirally thickened idioblasts in roots of *Stanhopea ebornea* (Link's spelling = *eburnea*). Other than these casual references, data is scant concerning *Stanhopea* stomatal complexes.

During normal development, members of this genus receive copious water. Thus, they should exhibit stomata indices not greatly different from one another. However, use of the Salisbury formula yields inconclusive data when calculating a baseline stomata index for individual species (see SI_{cells}: Table 1). With wide ranges reported by previous workers and the minimal date regarding this genus, a closer look at the formula was indicated.

The stomata index formula assumes that the leaf surface displays epidermal cells and stomata within a given area, and given the material with which Salisbury worked, this assumption is both obvious and quite correct. However, the literature is unclear regarding whether certain epidermal cells (trichomes and their flanking cells) are excluded, included as a unit, or counted as individual entities. Additionally, the literature is unclear whether stomatal subsidiary cells are counted separately or included as an entity of the stomatal complex. Thus, applying this as a general formula may not allow for other organs on the leaf surface, and results may be correct or merely the product of a mathematical error. In *Stanhopea* plants trichomes are present on both adaxial and abaxial leaf surfaces, with stomata only on the abaxial surface with the stomata are flanked by paracytic subsidiary cells. Thus, in order to arrive at a more accurate measure of the stomata index, the leaf surface space taken by these epidermal organs should be considered. However, counting the number of stomata within a given area leads to estimates when several of these organs overlap the borders of the selected area, and thus inaccuracies can be introduced in the number of stomata actually represented in that area of the leaf surface. Additionally, the basic formula does not clearly consider variances in the sizes of subsidiary cells.

METHODS AND MATERIALS

Photomicrographs of the leaf surface were taken and a random sample (N = 25) of adaxial cells, abaxial cells, stomata guard cells, and individual

subsidiary cells was measured and the mean of each is shown in the table. Although the number of cells varies, the adaxial leaf area is equal to its abaxial area. Hence this identity can be expressed, using areas instead of numbers of cells, as shown in formula (1):

(1) $Ad_{cells} + Ad_{trichomes} = Ab_{cells} + Ab_{trichomes} + \text{Stomata} + \text{Subsidiary Cells}$

The number of trichomes in these *Stanhopea* plants number only one or two in a standard area, resulting in a negligible difference in the adaxial and abaxial areas. Thus their effect has been assumed as equal and they are dropped from these calculations. However, where trichomes are numerous or adaxial and abaxial quantities differ greatly, their numbers and areas should be included in calculations. With this modification, the identity is expressed as in formula (2).

(2) $Ad_{\text{cells area}} = Ab_{\text{cells area}} + \text{Stomata}_{area} + \text{Subsidiary Cells}_{area}$

Using the random sample of 25 epidermal cells, another modification is necessary to arrive at an accurate number of stomata complexes. As each set of *Stanhopea* guard cells is supported by two paracytic subsidiary cells, the identity becomes as shown in formula (3).

(3) $25\ (Ad_{\text{cells area}}) = 25\ (Ab_{\text{cells area}}) + X\ [\text{Stomata}_{area} + 2\ (\text{Subsidiary Cells}_{area})]$.

Entering numerical values (e.g. C#01, *S. saccata*) gives:

(3a) $25\ (3097.40) = 25\ (2219.28) + X\ [1476.94 + 2\ (2024.48)]$.

Thus the number of stomata (X) within the specified area = 3.973.

Inserting this value into the modified Salisbury formula gives the following:

$$SI = \frac{S}{E+S} = \frac{\#St[areaSt+2(areaSS)]}{E+\#St[areaSt+2(areaSS)]} = \frac{3.973[1476.94+2(2024.48)]}{25(2219.28)+3.973[1476.94+2(2024.48)]} = \frac{21.954}{77436.4}$$

and $SI = 0.284$

Merely counting the number of stomata complexes within a specified area introduced inaccuracies due to variances in guard cell sizes, as well as stomata overlapping area boundary lines, and exclusion of the areas of subsidiary cells. These counts produced false stomata indices as shown in the column headed SI Cell, and thus showed greater differences between plants of the same species as well as of different species of the same genus. Using the number of abaxial cells per square millimeter (e.g. C01 *S. saccata* = 450.6), and the number of stomata (3.973) in the classic formula gives the index as shown in formula (4):

(4) $$SI = \frac{S}{E+S} \times 100 = \frac{3.973}{450.6 + 3.973} \times 100 = 0.874$$

RESULTS

TABLE 1. Summary of 30 *Stanhopea* species. Ad Area: mean adaxial cell surface area; Ab Area: mean adaxial cell surface area; Ab Cells: abaxial cells p/sq mm; # Stoma: mean number of stoma p/sq mm; GC Area: mean area of guard cells; SS Area: mean subsidiary cell area; SI Cell: index per Salisbury formula; SI Area: index per modified formula

C# Species	Ad Area	Ab Area	Ab Cells	# Stoma	GC Area	SS Area	SI Cell	SI Area
01 *saccata*	3097.40	2219.28	450.60	3.973	1476.94	2024.48	.874	.284
02 *saccata*	3040.90	2219.09	450.64	3.715	1264.14	2133.04	.817	.270
03 *insignis*	4769.92	3247.80	307.90	6.567	1993.45	1900.39	2.088	.319
04 *hernandezii*	2782.83	2096.11	477.07	4.202	1291.47	1397.28	.873	.247
05 *oculata*	4123.24	2574.44	388.43	9.18	1476.80	1581.22	3.055	.376
06 *oculata*	4173.52	2535.24	394.44	8.50	1554.22	1632.02	2.110	.393
07 *tigrina*	4259.11	2857.66	349.94	6.774	919.92	2126.08	1.899	.329
08 *graveolens*	3380.38	2372.18	421.55	3.698	1467.43	2859.74	.870	.298
09 *graveolens*	3288.62	2309.55	432.98	5.93	1518.74	1400.99	1.351	.297
10 *martiana*	3744.41	2700.86	370.25	5.837	1215.20	1627.29	1.552	.288
11 *intermedia*	2694.32	2060.25	485.38	2.888	1318.93	2085.25	.591	.235
12 *wardii*	4603.10	3546.56	281.96	4.799	1302.88	2100.33	1.673	.230
13 *wardii*	4594.98	3526.38	283.58	6.004	1568.32	1440.43	2.073	.233
14 *wardii*	4660.02	3570.86	280.04	5.092	1343.93	2001.75	1.786	.234
15 *nigroviolacea*	3129.27	2263.86	441.72	4.982	1137.87	1602.38	1.115	.277
16 *insignis*	4780.59	3251.88	307.51	7.176	1287.34	2019.38	2.290	.319
17 *costaricensis*	3605.70	2648.90	377.52	4.412	1427.67	1997.20	1.155	.265
18 *pozoi*	4891.37	2991.96	334.23	10.542	1520.17	1492.10	3.058	.388
19 *shuttleworthii*	4377.18	2808.95	356.01	7.304	1334.66	2016.36	2.010	.358
20 *tricornis*	4532.35	3555.51	281.25	5.077	1181.80	1813.87	1.773	.216
21 *reichenbachiana*	5623.69	3766.63	265.49	11.819	1337.21	1295.46	4.262	.330
22 *eburnea*	4498.03	3726.32	268.36	3.157	1419.09	2346.20	1.163	.172
23 *ecornuta*	3837.05	2756.76	362.75	4.838	1450.68	2065.54	1.316	.282
24 *jenischiana*	4368.36	2899.41	344.90	5.508	1360.17	2653.54	1.572	.336
25 *grandiflora*	4816.54	3714.49	269.22	8.525	1222.81	1004.59	3.069	.229
26 *tigrina*	4220.15	2856.17	350.12	6.258	1128.68	2159.94	1.756	.323
27 *candida*	4856.62	2790.16	358.40	11.420	1355.76	1584.00	3.088	.425
28 *connata*	4713.00	3213.80	311.16	6.833	1477.35	2003.84	2.149	.318
29 *haselowania*	4215.97	3556.07	281.21	2.295	1471.04	2858.26	.810	.157
30 *impressa*	4307.50	3119.53	320.56	10.26	1072.09	1228.61	3.101	.276

DISCUSSION

Although the area method involves more calculations, it provides a more accurate indication of the stomata and subsidiary cells within a given leaf surface area. While the number of cells varies on both the adaxial and abaxial leaf surfaces, obviously the *areas* of the leaf surfaces should be equal as should the sums of the areas of the organs on each surface. The literature is silent concerning subsidiary cells in calculations of the stomata index, but these cells occupy significant areas of the abaxial leaf surface in *Stanhopea* as well as other monocotyledons. In *Stanhopea*, stomata and subsidiary cells occur only on the abaxial leaf surface, with the guard cells of all species surveyed accompanied by subsidiary cells of varying areas. However, this does not

hold true for all other plants or even all members of the Orchidaceae, and particular attention should be paid to modification of the general formula in order to properly reflect the particular physiological condition of the plants being analyzed.

Previous workers have reported wide ranges of the stomata index in and between plants of the same species. However, in this survey of 50% of this genus the stomata index is not significantly different for plants of the same species, nor is a wide range observed between different species. It is suggested that calculations of the stomata index include subsidiary cells and any other types included in the count, as well as noting them as such. It is also suggested that the stomata index formula be modified to consider the areas of all components rather than being calculated by merely a count of unspecified epidermal cells and only the guard cells of stomata.

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